# Fast genotyping from plant samples

## User manual

NucleoType Plant PCR

NucleoType Seed PCR

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# 1 Components

## 1.1 Kit contents

| | NucleoType Plant PCR | | |
|---|---|---|---|
| **REF** | **25 preps 743202.25** | **100 preps 743202.100** | **500 preps 743202.500** |
| Plant Transfer Tool (PTT) | 25 pieces | 100 pieces | 500 pieces |
| NucleoType HotStart PCR Master Mix (2x) | 125 µL | 500 µL | 2 x 1250 µL |
| User manual | 1 | 1 | 1 |

| | NucleoType Seed PCR | | |
|---|---|---|---|
| | **25 preps 743203.25** | **100 preps 743203.100** | **500 preps 743203.500** |
| Lysis Buffer P | 12 mL | 60 mL | 250 mL |
| Liquid Proteinase K | 50 µL | 250 µL | 1250 µL |
| NucleoType HotStart PCR Master Mix (2x) | 125 µL | 500 µL | 2 x 1250 µL |
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## 1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- Primer for plant specific target of interest
- Water (PCR grade; for primer dilution and reaction fill-up)

Consumables

- Disposable pipette tips
- PCR tubes

Equipment

- Manual pipettes
- Thermoshaker or vortexer (for NucleoType Seed PCR)
- Personal protection equipment (lab coat, gloves, goggles)
- PCR cycler
- Gel electrophoretic equipment or Bioanalyzer® for analysis of generated amplicons

## 1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoType Plant PCR** kit / **NucleoType Seed PCR** kit before using these products.

All technical literature is available online at ***www.mn-net.com***.

Please contact technical service regarding information about any changes to the current user manual compared with previous revisions.

# 2 Product description

## 2.1 The basic principle

Many plant genotyping methods are based on DNA purification from plant material, followed by PCR amplification of genes of interest. However, DNA purification from plant material is a time consuming and elaborate process.

The **NucleoType Plant PCR** Kit / **NucleoType Seed PCR** Kit is designed for rapid plant typing experiments using plant leaf or seed material as sample, without the need to purify DNA.

For plant leaf material, the **NucleoType Plant PCR** Kit provides the patent pending Plant Transfer Tool (PTT) for sample take up, inhibitor inactivation, and transfer of the sample into the PCR. The sample harvest (pierce PTT into leaf) and transfer of plant material into the prepared PCR mix (short contact of PTT with PCR mix) takes less than five seconds. Due to the patent pending coating of the transfer tool, PCR inhibitors present in many plant materials are inactivated to a considerably extend during sample transfer.

A simple sample transfer with the PTT is not possible for hard plant samples. The **NucleoType Seed PCR** kit provides – instead of the PTT – the optimized Lysis Buffer P and Proteinase K for a simple sample preparation within a few minutes.

## 2.2 Kit specifications

**Table 1: Kit specifications at a glance**

| Parameter | NucleoType Plant PCR |
|---|---|
| Technology | Direct PCR: Transfer of plant leaf aliquot with Plant Transfer Tool (PTT) directly into PCR Mix |
| Format | 10 µL HotStart PCR (optional up to 50 µL) |
| Sample type | Plant leaf material from e.g., corn, soybean, wheat, *arabidopsis*, tobacco, cotton, grape wine, cress, as well as from kiwi, banana, and avocado fruit flesh |
| Preparation time | Sample preparation: < 1 min;<br>PCR cycling: 30–90 min (cycler and target size dependent) |
| Amplicon size | Up to 1000 bp |
| Analysis | Gel electrophoresis: Approx. 30 min (40 samples);<br>Bioanalyzer®: Approx. 40 min (12 samples) |

**Table 1: Kit specifications at a glance**

| Parameter | NucleoType Seed PCR |
|---|---|
| Technology | Simple sample preparation suitable for hot start PCR |
| Format | 10 µL HotStart PCR (optional up to 50 µL) |
| Sample type | Hard plant material like e.g., seeds from soybeen, wheat, corn, rice and tobacco, as well as from moss, fern leaf, and fir needle |
| Preparation time | Simple sample preparation in less than 5 min;<br>PCR cycling: 30–90 min (cycler and target size dependent) |
| Amplicon size | Up to 2000 bp |
| Analysis | Gel electrophoresis: Approx. 30 min (40 samples);<br>Bioanalyzer®: Approx. 40 min (12 samples) |

## 2.3 Handling, preparation, and storage of starting material

The kits are designed to perform genotyping from fresh plant material like leafs and seeds.

Plant material stored at 0–8 °C for several days or frozen material may also be used. However, kit performance with plant material stored for a long time might differ to the performance of fresh material.

## 2.4 Lysis, disruption, and transfer of sample material

In order to obtain reliable plant typing data, it is important to obtain a sufficient amount of DNA in a form suitable to serve as template for subsequent PCR amplification.

The **NucleoType Plant PCR** kit provides the Plant Transfer Tool (PTT) enabling an easy and patent pending sample uptake from plant leafs and transfer into the PCR mix. A special sample lysis step is not required. Upon insertion of the PTT into the plant leaf material, plant cells are disrupted and sufficient DNA will adhere to the PTT. During transfer of the sample into the PCR, inhibitors are inactivated due to the special coating of the PTT considerably. After sample transfer, it is possible to store the PCR mix for up to 2 hours at 4 °C–37 °C, due to HotStart function of the NucleoType HotStart PCR Master Mix.

The **NucleoType Seed PCR** kit contains Lysis Buffer P and Proteinase K enabling a simple sample preparation within in few minutes for e.g., plant seeds. The lysis procedure releases DNA in sufficient amount and quality to serve as template for the subsequent PCR.

# 3 Storage conditions and preparations of working solutions

**The NucleoType Plant PCR and NucleoType Seed PCR kit should be stored upon arrival at + 4 °C or -20 °C (recommended).**

The kit is stable for at least 12 months when stored at this temperature. The kit can be shipped at ambient temperature (18–25 °C) for up to 3 months. Short time exposure (up to 14 days) at temperatures up to 37 °C is tolerable.

Store all kit components at +4 °C or -20 °C (recommended) upon arrival and after first time usage.

Lysis Buffer P, Liquid Proteinase K, and NucleoType HotStart PCR Master Mix (2x) are ready to use.

Store NucleoType HotStart PCR Master Mix in the dark, e.g. within the product box in a freezer (- 20 °C; recommended) or fridge (+ 4 °C). Avoid prolonged exposure of the mix to light. Setting up PCR at average laboratory illumination is tolerable. Do not expose the mix to direct sunlight.

Prepare a primer mix according to the recommended concentration per primer as described in section 5.3.

# 4 Safety instructions

Use the product according to the user manual.

The product does not contain components requiring GHS hazard or precaution phrases.

# 5 Protocols

## 5.1 Plant typing with plant leaf material using the Plant Transfer Tools (PTT)

**Prepare sample**

*Provide plant material.*

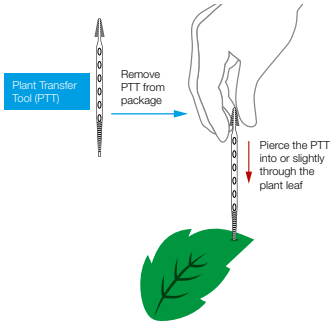
**A: *In situ* sampling (on-site sampling, greenhouse sampling)**

Sampling may be executed *in situ,* i.e. the plant to be typed or parts thereof are not transferred to the lab.

**1 Prepare Plant Transfer Tool**

At the plant growing site, withdraw a Plant Transfer Tool (PTT) from the bag, grabbing it firmly on the brushed end.

*Note: Do not touch the tip of the PTT with your finger or anything else except the sample in order to prevent any contamination and to prevent the active ingredient containing coating of the PTT tip to be wiped off.*



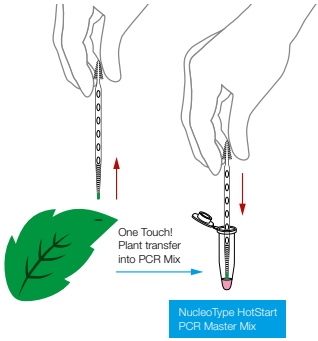

**2 Take up plant sample**

Pierce the PTT into, or slightly through the plant leaf. Optimally, pierce the PTT into the leaf while the leaf is backed by a solid to semi solid support. Do not pierce the PTT more than approximately 1–3 mm into/through the leaf. See section 7.1 Troubleshooting for additional recommendations.

**3 Transfer sample into the PCR**

Remove the PTT from the plant and dip the tip of the PTT briefly into the PCR tube (one touch, approximately 1 second, no stirring) containing the PCR mix, which was prepared in advance according to section 5.3. Alternatively it is possible to transport the PTT with adhered plant material (which is often almost invisible) to the lab, followed by plant sample transfer into the PCR tube (one touch, approximately 1 second, no stirring) containing the PCR mix.

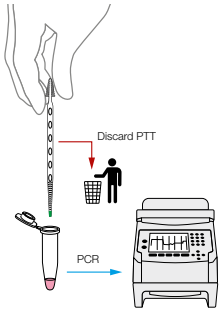
*Note: Transport of the PTT with adhered plant material may take up the 1.5 hours. It is important, that the PTT tip carrying plant material does not touch anything in order to avoid removal of surface coating. The PTT can be placed tip up in e.g., a tube rack.*




**4 Store sample or start PCR**

Discard the PTT and close the lid of the PCR tube.

*Note: After sample transfer, it is possible to store the PCR Mix for up to 2 hours in the dark at 4 °C–37 °C, due to HotStart function of the NucleoType HotStart PCR Master Mix. Avoid prolonged exposure of the PCR to light – do not expose to direct sunlight!*




## B: *Ex situ* sampling (lab sampling, sampling from cut off leaf material)

Plants, leafs or parts of leafs can be transported to the lab for sampling.

**1 Prepare Plant Transfer Tool**

Withdraw a Plant Transfer Tool (PTT) from the bag grabbing it firmly on the brushed end.

*Note: Do not touch the tip of the PTT with your finger or anything else except the sample in order to prevent any contamination and to prevent the active ingredient containing coating of the PTT tip to be wiped off.*

**2 Take up plant sample**

Pierce the PTT into, or slightly through the plant leaf. Optimally, pierce the PTT into the leaf while the leaf is backed by a solid to semi solid support. Do not pierce the PTT more than approximately 1- 3 mm into/through the leaf. See section 7.1. Troubleshooting for additional recommendations.

**3 Transfer sample into the PCR**

Dip the tip of the PTT briefly into the PCR (one touch, approximately one second, no stirring), which was prepared in advance according to section 5.3. Close the lid of the PCR tube and start PCR cycling.

Store sample or start PCR according to section 5.1 A.

## 5.2 Plant typing of seeds using Lysis Buffer P and Proteinase K

**1 Prepare final Lysis Buffer P**

Add **100 µL–500 µL Lysis Buffer P** into a lysis tube (1.5 mL or 2.0 mL; not provided).

Add **0.5 µL Liquid Proteinase K** per 100 µL Lysis Buffer P.

*Note: If several samples are processed at a time, a premix of Lysis Buffer P and Liquid Proteinase K can be prepared. Such a premix is stable at least 1 h at room temperature (18–25 °C).*

**2 Incubate sample and release DNA**

**Place a plant seed sample into the final lysis buffer.**

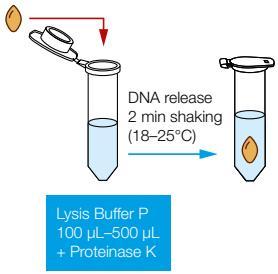
Recommendations for adequate final lysis buffer volume

- Small seeds like *arabidopsis*, wheat, rice: 100 µL Lysis Buffer P
- Medium seeds like soybeen: 200 µL Lysis Buffer P
- Large size seed like corn or cotton: 500 µL Lysis Buffer P

**Incubate 2 min** shaking at room temperature (18–25 °C; DNA release step).

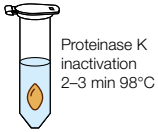
*Note: If continuous shaking is not possible, a motionless incubation can be performed. However an initial shaking is required.*

*Note: Incubation times of 1–5 min are possible and show similar results.*



**3 Deactivate Proteinase K**

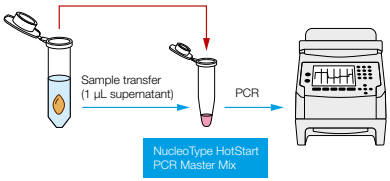
**Incubate 2–3 min at 98 °C** (Proteinase K inactivation step) while shaking. If continuous shaking is not possible, a motionless incubation can be performed.




**4 Transfer sample into the PCR**

**Use 1 µL lysate as template** for subsequent PCR.

*<u>Note:</u> For storage of the lysate, the plant seed has to be removed from the lysate! The lysate can then be stored up to 6 months at +4° C or -20 °C. Even storage at room temperature (18–25 °C) is possible for up to 6 weeks. Repeated freeze-thaw cycles are unproblematic.*



## 5.3 Reaction setup for 10 µL PCR (single-plex or duplex)

A final PCR with 10 µL volume is the recommended standard reaction volume for the **NucleoType Plant PCR** and **NucleoType Seed PCR** kits. Due to the hot start technology of this product, the reaction setup can be performed at room temperature (18–25 °C).

Per reaction combine the following

- 5 µL NucleoType HotStart PCR Master Mix (2x).
- 5 µL primer mix (each target primer with a concentration of 0.4 µM within the 5 µL primer mix, resulting in a final concentration of 0.2 µM in the PCR per primer).

Final PCR mix: 10 µL final PCR volume, ready to receive the plant sample directly from the Plant Transfer Tool (PTT) or 1 µL from the seed lysate.

*Note: The addition of silicone oil is not necessary and will impair removal of the liquid after the reaction. Therefore, the addition of silicone oil is not recommended.*

*Note: If desired, the final PCR volume can be scaled up by increasing all components proportionally.*

## 5.4 PCR cycling parameters

Cycling conditions are depending on primer, target length, and PCR cycler setup. For several primer pairs with $T_m$ ranging from 40 °C to 75 °C the following PCR programs have been used successfully.

| **PCR program 1 (three step program for typical endpoint PCR cycler)** | | | |
|---|---|---|---|
| Initial denaturation | 95 °C | 2 min | 1 cycle |
| Amplification: | 95 °C<br>40–75 °C*<br>72 °C | 15 s<br>20 s**<br>60 s** | 40 cycles |
| Extension | 72 °C | 1 min | 1 cycle |
| Cooling | 4 °C | | |
| Total time | | Approx. 70–100 min (total run time is annealing temperature and machine dependent) | |

*Note: For initial testing annealing time of 20 s and extension time of 60 s is recommended. For amplification of fragments smaller 1000 bp and / or for amplifications with a PCR machine with slow ramp rates (e.g. 2 °C/s) annealing / and extension time may successively be reduced (e.g. to 15 s annealing and 15 s extension). PCR machines with fast ramp rates (e.g. 5°C/s) may not be reduced as much as for slow ramping machines.*

* Optimal annealing temperature is primer dependent and has to be determined empirically. A good starting point for testing is 50 °C–55 °C. Optimally, a good annealing temperature for primer of your choice is determined with a temperature gradient cycler.

** For initial testing an annealing time of 20 seconds and an extension time of 60 seconds is recommended!

| PCR program 2 (two step program for typical end point PCR machines) | | | |
|---|---|---|---|
| Initial denaturation | 95 °C | 2 min | 1 cycle |
| Amplification | 95 °C<br>60–72 °C* | 15 s<br>60 s** | 40 cycles |
| Extension | 72 °C | 1 min | 1 cycle |
| Cooling | 4 °C | | |
| Total time | | Approx. 60–70 min (total run time is annealing / elongation temperature and machine dependent) | |

*Note: For initial testing annealing / extension time of 60 s is recommended. For amplification of fragments smaller 1000 bp and / or for amplifications with a PCR machine with slow ramp rates (e.g. 2° C/s) annealing / and extension time may successively be reduced (e.g. to 15 s annealing and 15 s extension). PCR machines with fast ramp rates (e.g. 5 °C/s) may not be reduced as much as for slow ramping machines.*

| PCR program 3 (e.g., LightCycler® 1.5 in glass capillary) | | | |
|---|---|---|---|
| Initial denaturation | 95 °C | 2 min | 1 cycle |
| Amplification | 95 °C<br>40–75 °C***<br>72 °C | 15 s<br>15 s<br>30 s | 40 cycles |
| Extension | 72 °C | 1 min | 1 cycle |
| Cooling | 20 °C | | |
| Total time | | Approx. 30–60 min (total run time is annealing / elongation temperature and machine dependent) | |

*Note: The LightCycler® is used herein solely as a fast cycling instrument, but not for quantitative PCR!*

*Note: : It is recommended to target sequences not exceeding 500 bp in glass capillaries.*

* The optimal annealing / extension time is primer dependent. Only primers with melting temperature above 60 °C are recommended for this program.

**For initial testing an annealing / extension time of 60 seconds is recommended!

*** Optimal annealing temperature is primer dependent and has to be determined empirically. A good starting point for testing is 50 °C. Optimally, a good annealing temperature for primer of your choice is determined with a temperature gradient cycler.

# 6 Analysis of PCR products

The PCR products (amplicons) can directly be analyzed using the following methods.

There is no need to add loading dye for gel electrophoresis, because the PCR mix already contains a dye and suitable density.

There is no need to perform a proteinase digestion step prior to analysis of the amplicons.

- Gel electrophoresis: Apply the total PCR reaction onto an e.g., 1–2 % agarose gel for analysis.
- Dye migration in

  1 % agarose gel: Approximately as 600 bp fragment

  2 % agarose gel: Approximately as 350 bp fragment
- Bioanalyzer® (Agilent): Use 1 µL with e.g. the Agilent DNA 1000 Kit.

# 7 Appendix

## 7.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Reduction of PCR volume | *Reduction of initial 10 µL PCR volume during cycling*<br>• Depending on the PCR tube size and quality, the initial 10 µL setup volume might shrink to approximately 8 µL during cycling. This is acceptable and does not impair typing performance. If volume reduction is more pronounced, use a smaller and / or tighter reaction tube. |
| To little or no amplicon detected | *Unfavorable primer selection*<br>• Make sure that the primers are selected well and are able to amplify the desired target from 1–10 ng of purified genomic DNA. Test different primer annealing temperatures.<br>*Unfavorable storage conditions*<br>• Store NucleoType HotStart PCR Master Mix in the dark, e.g. within the product box in a freezer (- 20 °C; recommended) or fridge (+ 4 °C). Avoid prolonged exposure of the mix to light. Setting up PCR at average laboratory illumination is tolerable. Do not expose the mix to direct sunlight.<br>*Insufficient uptake of sample material*<br>• For very small leafs, its possible to circumnvent insufficient sample uptake by using a support pad. The leaf should be backed up by a solid to semi solid support (e.g. rubber pad, piece of disposable plastic sheet) and the PTT is pierced into the leaf.<br>• For common leafs like tobacco or wine leaf, the PTT in inserted less than approximately 1 mm. Do not pierce the PTT in or through the plant leaf for more than 1–3 mm.<br>*Too much sample material in PCR*<br>• Make sure to transfer plant leaf material with the Plant Transfer Tool (PTT) into the PCR. Typically, plant leaf material adhering to the PTT is faintly visible. Do not transfer bits and pieces of plant material.<br>*No Plant Transfer Tool (PTT) used.*<br>• The substitution of the PTT by a common toothpick may cause reaction failure. The PTT is coated with an active component which inactivates several PCR inhibitors which are commonly present in plant leafs.<br>*PTT not handled with care*<br>• The PTT tip is coated with an active ingredient, which might be wiped off, in case of too much contact of the tip with surfaces or fingers. Do not touch the PTT tip – grab it firmly on the brushed side! |

| Problem | Possible cause and suggestions |
|---|---|
| To little or no amplicon detected | *PTT uses as a stirrer*<br>• Do not “stir in” the sample into the PCR – a short, approximately 1 second dip in of the PTT tip into the PCR mix without stirring is sufficient for sample transfer!<br>*Unfavorable PCR program*<br>• Try to adjust annealing temperature and time as well as extension time. Note that PCR machines with rapid ramp rates require longer annealing and extension times than PCR machines with slow ramp rates, because there is less amplification time for the polymerase during ramping! |
| Amplicon does not have the correct size | *Primer selection*<br>• Make sure that the primers are selected well and are able to amplify the desired target from 1–10 ng or purified genomic DNA. |
| Amplicon number is not correct | *Sensitivity of analysis method*<br>• Make sure that the analysis method has enough resolving power to discriminate the two different sizes of DNA fragments in case of a duplex PCR.<br>• Use Bioanalyzer® instead of gel electrophoreses or increase electrophoresis time or gel concentration.<br>• Make sure that both primer pairs have a similar amplification efficiency. If this is not the case, titrate down the primer pair yielding an amplicon (use a smaller concentration for this primer pair). |

## 7.2 Ordering information

| Product | REF | Pack of |
|---|---|---|
| NucleoType Plant PCR | 743202.25 / 100 / 500 | 25 / 100 / 500 reactions |
| NucleoType Seed PCR | 743203.25 / 100 / 500 | 25 / 100 / 500 reactions |

## 7.3 Product use and restriction / warranty

NucleoType Plant PCR and NucleoType Seed PCR kit components were developed, designed and sold for research purposes only. They are suitable for in vitro uses only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL’s sole obligation and the customer’s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

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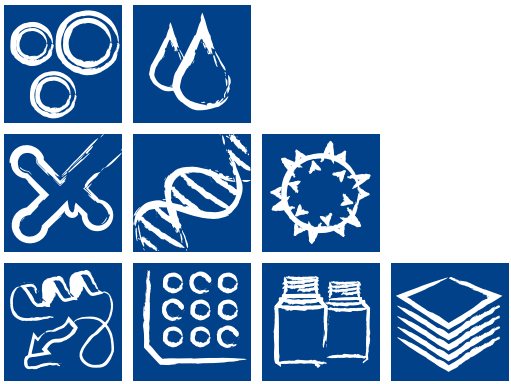


**Plasmid DNA**

**Clean up**

**RNA**

**DNA**

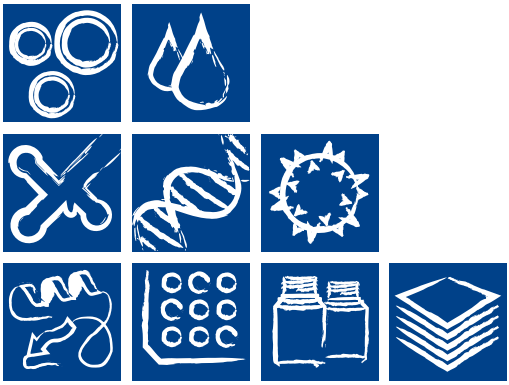
**Viral RNA and DNA**

**Protein**

**High throughput**

**Accessories**

**Auxiliary tools**



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