Fast genotyping from mouse samples

User manual

NucleoType Mouse PCR

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MACHEREY-NAGEL
www.mn-net.com

Contact MN

Germany and international
MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

Technical Support Bioanalysis
Tel.: +49 24 21 969-270
E-mail: tech-bio@mn-net.com

USA
MACHEREY-NAGEL Inc.
2850 Emrick Blvd. · Bethlehem, PA 18020 · USA
Tel.: +1 484 821 0984
Toll-free: 888 321 6224 (MACH)
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

France
MACHEREY-NAGEL SARL à associé unique
1, rue Gutenberg · 67722 Hoerdt · France
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

Switzerland
MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoType Mouse PCR		
REF	**25 preps 743200.25**	**100 preps 743200.100**	**500 preps 743200.500**
Lysis Buffer M	12 mL	12 mL	60 mL
Liquid Proteinase K	50 µL	50 µL	250 µL
NucleoType HotStart PCR Master Mix (2x) (containing polymerase, dNTPs, buffer, enhancer, stabilizer)	125 µL	500 µL	2x 1250 µL
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1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

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

Consumables

- Disposable pipette tips
- 1.5 mL or 2.0 mL microcentrifuge tube (for lysis)
- PCR tubes

Equipment

- Manual pipettes
- Centrifuge for microcentrifuge tubes (optional)
- Shaker (for lysis, recommended)
- Thermal heating block recommended (for Proteinase K inactivation), or heated water bath
- Personal protection equipment (lab coat, gloves, goggles)
- PCR machine

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the NucleoType Mouse PCR kit before using this product.

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

2 Product description

2.1 The basic principle

Many mouse genotyping methods are based on PCR amplification of genes of interest from tail biopsies or ear punches. The NucleoType Mouse PCR kit is designed for rapid mouse typing experiments and covers these standard sample materials including even blood and animal friendly hair samples.

Hair samples can be easily collected causing minimal discomfort to the animal and do not require anesthesia. Thus, the NucleoType Mouse PCR kit enables animal friendly and time saving mouse genotyping.

A simple sample preparation within one tube is combined with a fast PCR. Mouse samples (tail clipping, ear punch, blood, or fur hair) are sujected to a quick lysis in a small volume and short time (<5 min). For optimal results an aliquot from the lysate is used as template for PCR typing. The enzyme mix enables cycling times of less than one hour. Analysis of the amplicons can be performed by gel electrophoresis or Bioanalyzer®. The reactions contain a dye and are ready to load directly on the agarose gel. There is no need to add a dye in order to assist application of the amplicon onto the gel. The dye of the NucleoType Mouse PCR kit is compatibel with Bioanalyzer® (Agilent DNA 1000 Kit). Using a fast cycler, simple sample preparation and the subsequent PCR can be performed in less than one hour. In addition the NucleoType Mouse PCR kit offers an alternative direct PCR protocol allowing PCR directly from unpurified and undiluted samples.

2.2 Kit specifications

Kit specifications at a glance	
Parameter	NucleoType Mouse PCR kit
Technology	Simple sample preparation suitable for hot start PCR and direct PCR
Format	20–100 µL lysate; 10 µL PCR (optional 5–50 µL)
Sample type	Mouse tail clipping, mouse ear punch, mouse blood (fresh or frozen EDTA, citrate, heparin, or untreated), mouse hair
Sample amount	1 mm diameter ear punch, 1 mm outer tail clipping, or small tuft of hair (approximately 3–30 hairs), or 1 µL mouse blood
Typical yield	Strong bands in agarose gel electrophoresis, approximately 100 ng to 600 ng amplicon per 10 µL PCR (primer and target dependent)
Amplicon size	Up to 1000 bp
Preparation time	Simple sample preparation in less than 5 min PCR cycling: 30–90 min (cycler and target size dependent)
Analysis	Gel electrophoresis: Approx. 30 min (40 samples); Bioanalyzer®: Approx. 40 min (12 samples)

2.3 Handling, preparation, and storage of starting materials

The kit is designed to perform genotyping on the following sample materials: Mouse tail clipping, mouse ear punch, mouse hair, and mouse blood.

Fresh or frozen material can be used.

Respect your local animal welfare regulations when choosing, harvesting and handling mouse samples!

2.4 Lysis and disruption of sample material

In order to obtain reliable mouse typing data, it is important to obtain sufficient amount of DNA in a form suitable to serve as template for subsequent PCR amplification. The NucleoType Mouse PCR kit contains an optimized Lysis Buffer M for sufficient DNA release within a short 2 minute lysis step. For optimal PCR results we recommend to use an aliquot of this lysate as template for the PCR typing. Optional, the NucleoType Mouse PCR kit offers an alternative direct PCR protocol allowing PCR directly from unpurified and undiluted samples (see section 5.1 and 5.2).

3 Storage conditions and preparations of working solutions

The NucleoType Mouse PCR kit should be stored upon arrival at +4 °C or -20 °C. The kit is stable for at least 12 month when stored at this temperature. The kit can be shipped at ambient temperature for up to up to 3 month. Short time exposure (up to 14 days) at temperatures up to 37 ° is tolerable.

After first time usage, store all kit components at +4 °C or -20 °C.

The Lysis Buffer M, 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 containing primer for your target of interest. Recommended concentration per primer: See section 5.3 and 5.4.

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 Mouse typing with tail clip, ear punch, or hair

Lysis protocol (recommended)

1 Prepare final Lysis Buffer

Add **20 µL Lysis Buffer M** into a lysis tube (1.5 or 2.0 mL; not provided).

Add **0.5 µL Liquid Proteinase K** into the Lysis Buffer M.

Note: If several samples are processed at a time, a premix of Lysis Buffer M and Liquid Proteinase K can be prepared, e.g., 200 µL Lysis Buffer M with 5 µL Liquid Proteinase K. Such a premix is stable for at least 1 h at room temperature (18–25 °C).

2 Incubate and lyse sample

Place a **mouse typing sample** into the lysis tube

Mouse tail clip: Approximately 1 mm from the outer end of the tail.

Mouse ear punch: 1 mm in diameter ear punch. Use e.g. a biopsy punch 1 mm such as PF49101 Disposal Biopsy Punches with Plunger-System (pmf Medical).

Note: Ear punches with a smaller diameter (e.g., 0.3 mm) are hard to handle and placing of such small samples into the Lysis Buffer M may be imprecisely. Therefore such small samples are not recommended. Ear punches with a diameter larger than approximately 1.5 mm should not be used as they might overload the reaction.

Mouse hair: Place one tuft of hair (approximately 3–30 fur hairs) into the lysis tube. Make sure to place the hairs at the bottom of the tube, preferably roots down! Short centrifugation of the closed lysis tube may help to spin down the hairs. A slant tweezer with a 1 x 3 mm flat area to grab the fur hairs is recommended.

Incubate 2 min shaking at ambient 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).

4 Store sample or prepare PCR reaction mixture

Centrifuge the lysate briefly in order to sediment debris.

Note: Typically, a centrifugation step is not necessary. Residual tissue might still be visible but this does not impair the subsequent reaction.

The lysate can be stored for at least 2 weeks at room temperature (18–25 °C), 2 month at 4 °C or 2 years at - 20 °C. Repeated freeze-thaw cycles are unproblematic.

Use 1 µL liquid of this lysate as template for the subsequent PCR.

Note: Take care not to transfer residual tissue pieces.

For the direct PCR protocol place the sample (1 mm diameter ear punch, 1 mm outer tail clipping, or small tuft of hair; approximately 3–30 hairs) directly into the PCR reaction (50 µL of volume). Make sure that the sample is covered by the PCR Mix.

5.2 Mouse typing with blood

Lysis protocol (recommended)

1 Prepare final Lysis Buffer

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

Add **0.5 µL Liquid Proteinase K** into the Lysis Buffer M.

Note: If several samples are processed at a time, a premix of Lysis Buffer M and Proteinase K can be prepared, e.g., 1000 µL Lysis Buffer M with 5 µL Liquid Proteinase K. Such a premix is stable for at least 1 h at room temperature (18–25 °C).

2 Incubate and lyse sample

Add **1 µL mouse blood (fresh or frozen EDTA, citrate, heparin or untreated) as typing sample** into the lysis tube.

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

Note: If continuous shaking is not possible, a motionless incubation can be performed, however an initial mixing 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).

4 Store sample or prepare PCR reaction mixture

Centrifuge the lysate briefly in order to sediment debris.

Note: Typically, a centrifugation step is not necessary. Residual blood might still be visible but this does not impair subsequent reaction.

The lysate can be stored for at least 2 weeks at room temperature (18–25 °C), 2 month at 4 °C or 2 years at - 20 °C. Repeated freeze-thaw cycles are unproblematic.

Use 1 µL liquid of this lysate as template for the subsequent PCR.

For the direct PCR protocol place the sample (1 µL of a 1:20 diluted blood sample) directly into the PCR reaction (50 µL of volume).

5.3 Reaction setup for single-plex 10 µL PCR

The 10 µL reaction is the recommended standard reaction volume for the NucleoType Mouse PCR kit. Due to the hot start technology of this product, the reaction set up can be performed at room temperature (18–25 °C).

Per reaction combine the following:

5 µL NucleoType HotStart PCR Master Mix (2x)

2 µL forward primer (stock concentration 1 µM or 1 pmol/µL)

2 µL reverse primer (stock concentration 1 µM or 1 pmol/µL)

1 µL of mouse sample lysate

→10 µL final PCR volume

A final concentration of 0.2 µM per primer is recommended.

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: Depending on the PCR tubes used, the initial 10 µL set up volume might shrink to approximately 8 µL due to evaporation during cycling. This has been taken into account and does not impair the reaction.

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

If **duplex PCR** is set up, adjust primer concentration to 0.2 µM as final concentration for each primer. Use e.g.,

1 µL forward primer (stock concentration 2 µM) for target one

1 µL reverse primer (stock concentration 2 µM) for target one

1 µL forward primer (stock concentration 2 µM) for target two

1 µL reverse primer (stock concentration 2 µM) for target two

5.4 Reaction setup for single-plex 5 µL PCR

Due to the hot start technology of this product, the reaction set up can be performed at room temperature (18–25 °C). Per reaction combine the following:

2.5 µL NucleoType HotStart PCR Master Mix (2x)

0.1 µL forward primer (stock concentration 10 µM or 10 pmol/µL)

0.1 µL reverse primer (stock concentration 10 µM or 10 pmol/µL)

1.0 µL of mouse sample lysate

1.3 µL water

→5 µL final PCR volume

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: Depending on the PCR tubes used, the initial 5 µL set up volume might shrink to approximately 4 µL due to evaporation during cycling. This has been taken into account and does not impair the reaction.

PCR - Reaction setup overview

Component	5 µL PCR rxn	10 µL PCR rxn (recommended)	50 µL PCR rxn (direct PCR) tissue	50 µL PCR rxn (direct PCR) blood
NucleoType HotStart PCR Master Mix (2x)	2.5 µL	5 µL	25 µL	25 µL
Forward primer	0.1 µL (conc. 10 µM or 10 pmol/µL)	2 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)
Reverse primer	0.1 µL (conc. 10 µM or 10 pmol/µL)	2 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)
Sample (see section 5.1 and 5.2)	1 µL lysate	1 µL lysate	mouse tail clip ear punch, fur	1 µL of 1:20 diluted mouse blood
H_2O	1.3 µL	-	5 µL	4 µL

5.5 PCR cycling parameters

Cycling conditions are depending on primer and PCR machine set up. For several primer pairs with primer T_m ranging from 40 °C to 75 °C the following PCR programs have been used successfully.

For amplicons from 50–1000 bp an initial extension time of approximately 60 seconds is recommended.

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.

PCR program 1 (three step program for typical endpoint PCR machines)			
Initial Denaturation	95 °C	2 min	1 cycle
Amplification	95 °C 40–75 °C* 72 °C	15 s 20 s 60 s	40 cycles
Extension	72 °C	1 min	1 cycle
Cooling	4 °C		
Total time		ca. 70–100 min (total run time is annealing temperature and machine dependent)	

PCR program 2 (Two step program for typical end point PCR machines)			
Initial Denaturation	95 °C	2 min	1 cycle
Amplification	95 °C 60–72° C*	15 s 60 s	40 cycles
Extension	72 °C	1 min	1 cycle
Cooling	4 °C		
Total time		ca. 66 min (machine dependent)	

*: 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.

PCR program 3 (e.g., LightCycler® 1.5, in glas capillary)			
Initial Denaturation	95 °C	2 min	1 cycle
Amplification	95 °C 40 - 75 °C* 72 °C	15 s 15 s 30 s	40 cycles
Extension	72 °C	1 min	1 cycle
Cooling	20 °C		
Total time		ca. 30–60 min (anneling temperature dependent)	

Note: The LightCycler® is used herein solely as a fast cycling instrument, but nor for quantitative PCR!

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

6 Analysis of PCR products

The PCR products (amplicons) can be directly analyzed by one of the following methods.

Gel electrophoresis: Apply the total PCR reaction onto a agarose gel (e.g. 1 % agarose) for analysis.

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

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

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 initial 10 µL PCR set up volume during PCR cycling	• Depending on tightness of PCR tubes used, the initial 10 µL PCR set up volume might shrink to approximately 8 µL. This is acceptable and does not impair typing performance. If volume reduction is even more pronounced, use a tighter reaction tube.
No amplicon detected	*Unfavorable primer selection.* • Make sure that the primer are selected well and are able to amplify the desired target from 1–10 ng of purified genomic DNA. Test different primer annealing temperatures. *Unfavorable storage conditions* • *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.* *Too much extract in PCR.* • Make sure to use at most 1 µL lysate as template per reaction. *Liquid Proteinase K was skipped or heat incubation step was skipped.* • Make sure to add Liquid Proteinase K to the Lysis Buffer M and make sure to incubate for at least 2 min at 98 °C in order to inactivate the Proteinase K. *PCR cycling conditions not optimal.* • Decrease annealing temperature. Test different primer annealting temperatures. Increase extension time. Increase number of cycles up to 40.
Too little amplicon yield	• Try to adjust annealing temperature and extension time
Amplicon does not have the correct size	• Make sure that the primers are selected well and are able to amplify the desired fragment from 1-10 ng of purified genomic template DNA.

Problem	Possible cause and suggestions
Two amplicons of different sizes are expected, but only one band is observed by agarose gel electrophoresis	• Make sure that the analysis method has enough resolving power to discriminate the two different sizes of DNA fragments. Use Bioanalyzer® instead of gel electrophoresis or increase electrophoresis time or gel concentration. • 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 Mouse PCR	743200.25	25 reactions x 10 µL
NucleoType Mouse PCR	743200.100	100 reactions x 10 µL
NucleoType Mouse PCR	743200.500	500 reactions x 10 µL

7.3 Product use restriction / warranty

NucleoType Mouse 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).

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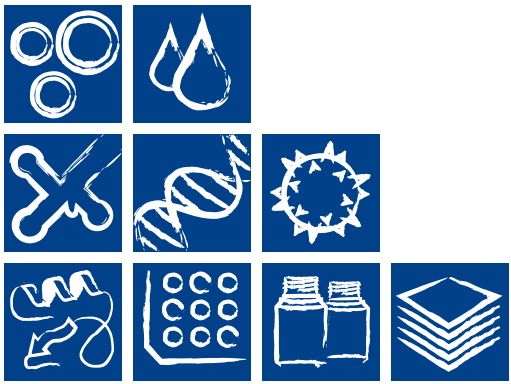
Please contact:
MACHEREY-NAGEL Germany
Tel.: +49 (0) 24 21 969-270
e-mail: TECH-BIO@mn-net.com



Plasmid DNA

Clean up

RNA

DNA

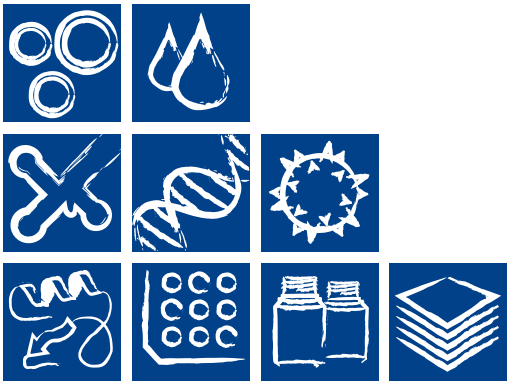
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



www.mn-net.com

MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

DE / International:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com