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Specific (first) PCR of the Leeselab 2-step PCR protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes how to perform the specific first step of the Leeselab 2-step PCR protocol in 10 µL reactions. In the first PCR step, universal primers (e.g. fwhF2/fwhR2n) are used toamplify the target sequence. There are up to 24 different first-step primers individually labeled with a 4 - 6 bp tag (see table in the material section). These are used to individually tag up to 24 different 96-Well PCR Plates. In addition to the specific primer and the tag, each of the primers contains a universal overhang, which is used to further amplify the product in the second PCR. In the second step of the 2-step-PCR, each well is tagged with an individual Index (1-96) enabling the identification of each sample (well) after library sequencing.

Guidelines

Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

Materials

Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable.

Chemicals:

Multiplex Mastermix 5x Hot-Start Multiplex PCR-Mastermix **Bio&SELL Catalog #**BS91.522.1000MPP
Forward Primer (universal), see Excel spreadsheet below
Reverse Primer (universal), see Excel spreadsheet below
PCR-grade water
Invitrogen UltraPure DNase/RNase-Free Distilled Water **Fisher Scientific Catalog #**11538646

Labware:

Micro-Twist-Top-Tube Micro-Twist-Top-Tube **Sarstedt Catalog #**72.693
PCR plate Hard-Shell 96-well Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601
PCR foil, DNase-/RNase-free PCR foil, DNase-/RNase-free **Sarstedt Catalog #**95.1993

Devices:

Vortexer
Centrifuge for SBS format microplates
Table centrifuge for tubes
Pipettes and Multichannel pipettes including pipette tips
Thermocycler compatible wth low-profile full-skirted PCR plates

Documents:

First_step_primer_Leeselab.xlsx 9.7KB

Protocol materials

Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601
Micro-Twist-Top-Tube **Sarstedt Catalog #**72.693
5x Hot-Start Multiplex PCR-Mastermix **Bio&SELL Catalog #**BS91.522.1000MPP
UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977023
PCR foil, DNase-/RNase-free **Sarstedt Catalog #**95.1993
Invitrogen UltraPure DNase/RNase-Free Distilled Water **Fisher Scientific Catalog #**11538646

Safety warnings

Reagents are potentially damaging to the environment. Dispose waste responsibly.

Before start

Make sure all materials are available before starting. Make sure all materials and samples are unfrozen.

Note

The protocol described here is designed for the use of PCR plates, but can also be done in tubes, strips, or any sufficient reaction vessel. The recommended shaking speeds are adjusted to the plates mentioned in the materials.

Preparations

26m

1 Calculate the required volumes for the PCR Mastermix using the Excel spreadsheet.

1m

Note

You can download the Exel spreadsheet here:
Mastermix_calculation.xlsx

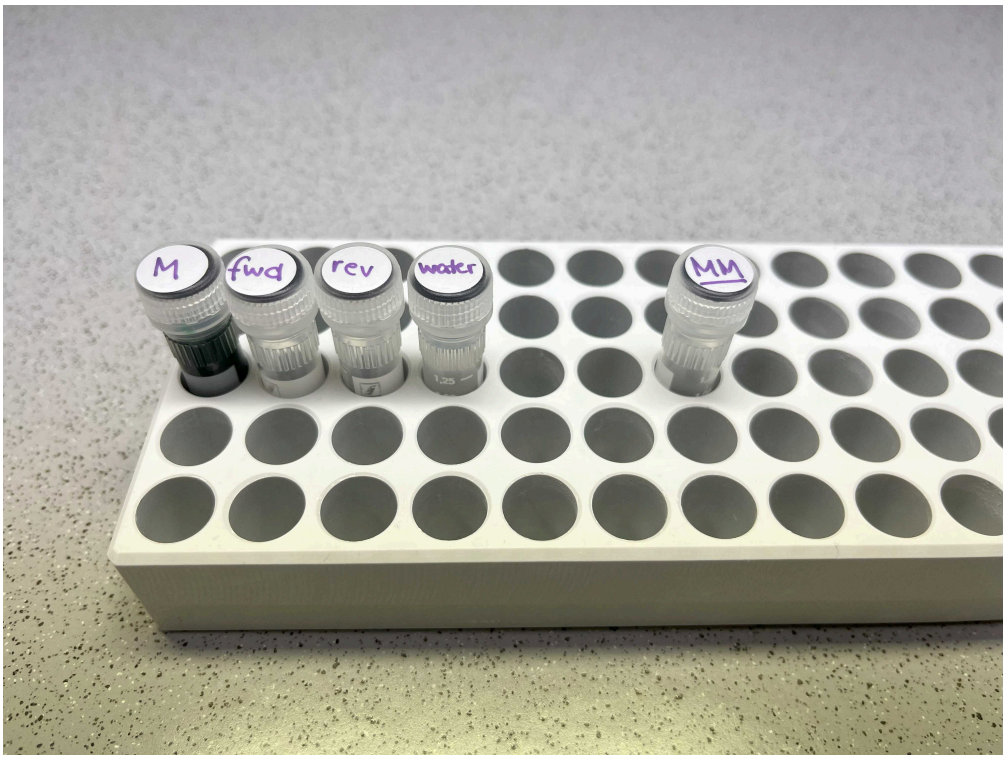
2 Prepare the workplace to ensure contamination-free handling.

2m

Workspace prepared for a right-handed person to work from left (pipette tips, samples) to right (waste). Samples and (unused) labware are placed in the back.

2.1 Label the Micro-Twist-Top-Tube **Sarstedt Catalog #**72.693 (e.g., "MM") and Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601 (e.g. project name, date, "1st_pcr", "A").

2m



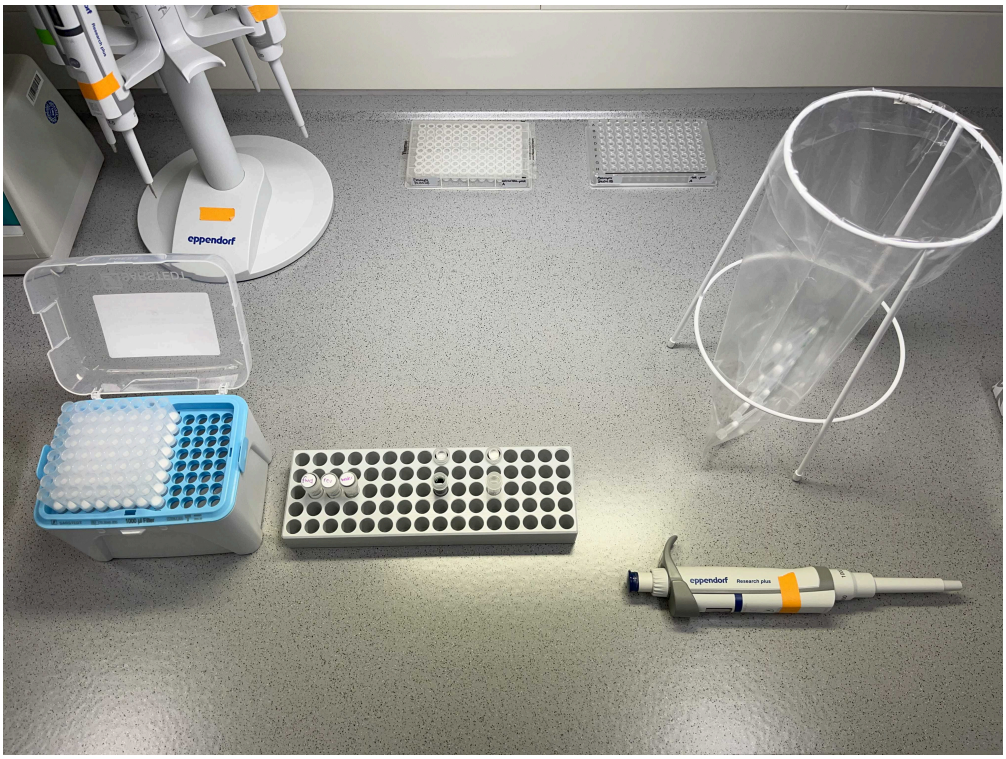
Multiplex Mastermix (M), forward (fwd) and reverse (rev) primer, PCR-grade water and PCR Mastermix (MM).

3 Briefly vortex and shortly spin down all samples (DNA extract) and required chemicals.

2m

4 Pipette the PCR Mastermix in a Micro-Twist-Top-Tube **Sarstedt Catalog #**72.693 containing the calculated volumes of 5x Hot-Start Multiplex PCR-Mastermix **Bio&SELL Catalog #**BS91.522.1000MPP , forward primer, reverse primer, and UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977023 .

3m



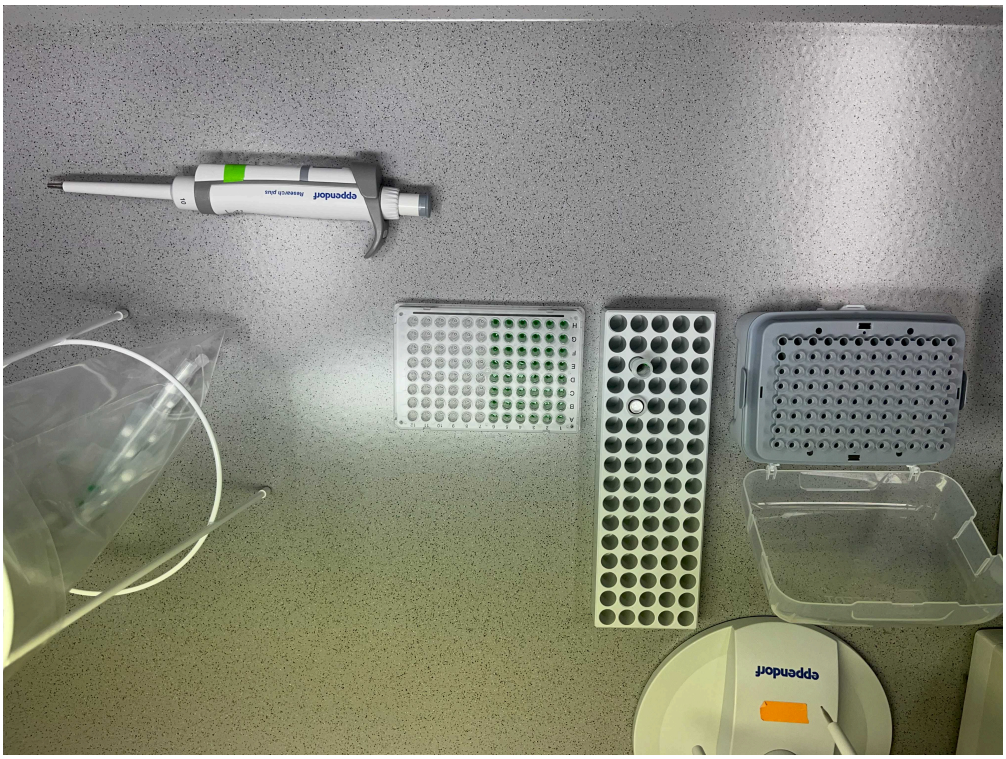
Preparation of the PCR Mastermix (MM).

Note

This step has to be performed separately per plate when using the tagging scheme mentioned above since per plate an individual combination of primers is used per plate.

4.1 Briefly vortex and shortly spin down the prepared PCR Mastermix. 1m

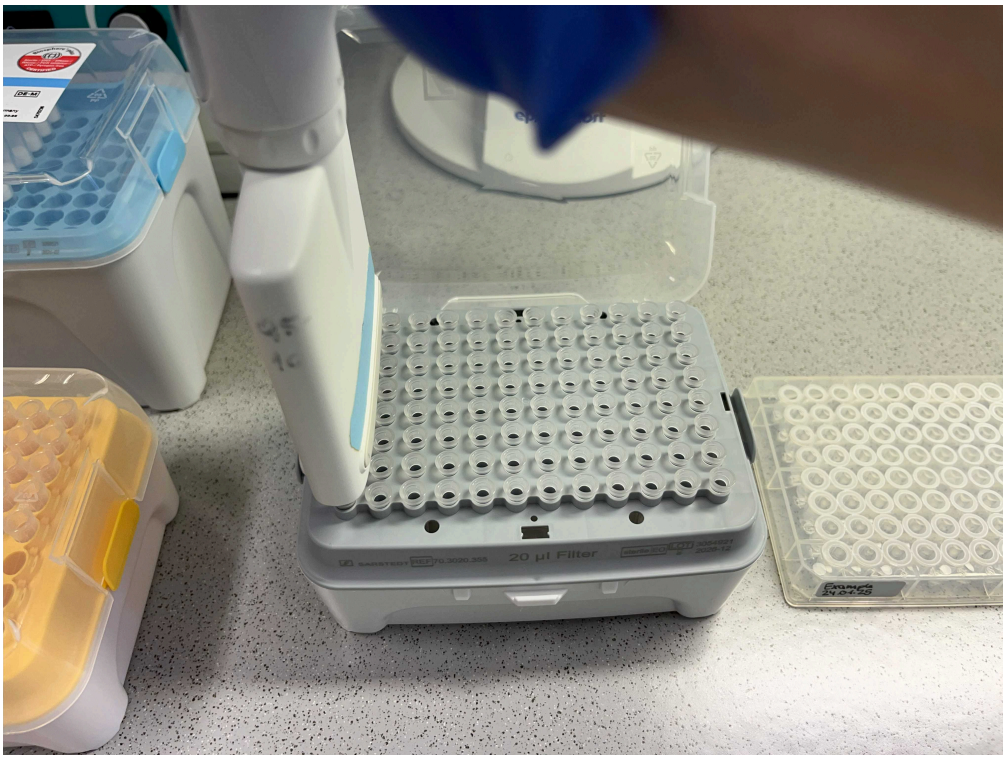
5 Distribute 9 µL per sample in each well used of the Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601. To speed up this step, a Multidispenser can be used. 8m



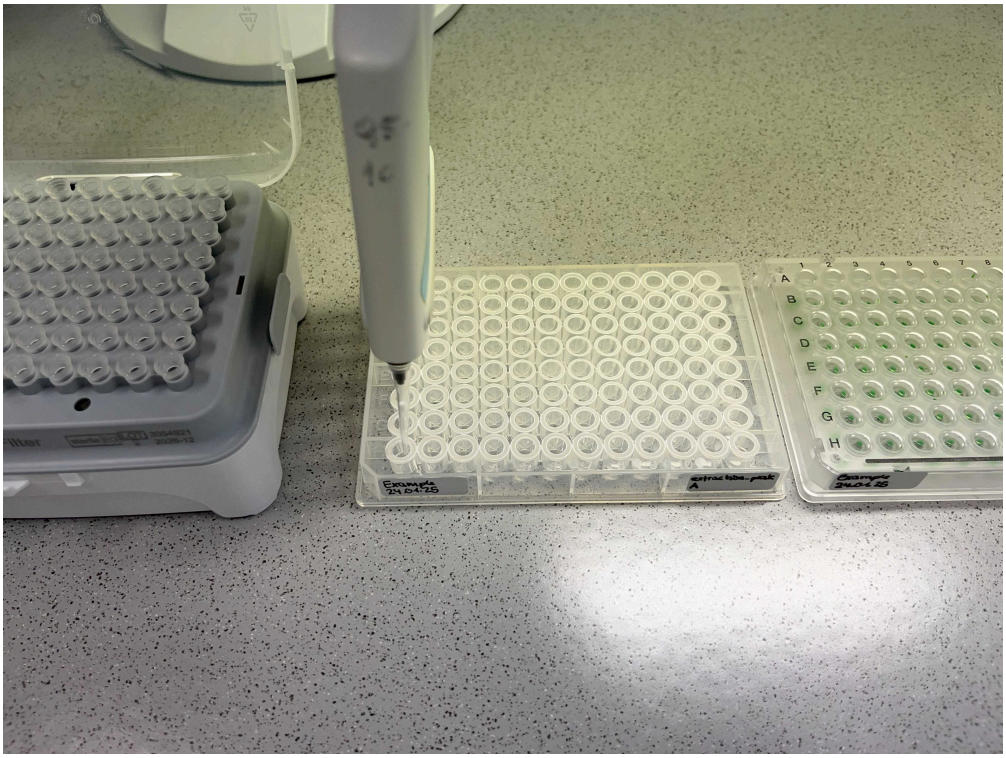
Distribution of the PCR Mastermix (MM) in all required wells.

6 Pipette 1 µL DNA extract per well. This step has to be done with a Multichannel pipette to ensure correct assignment and minimize the contamination risk.

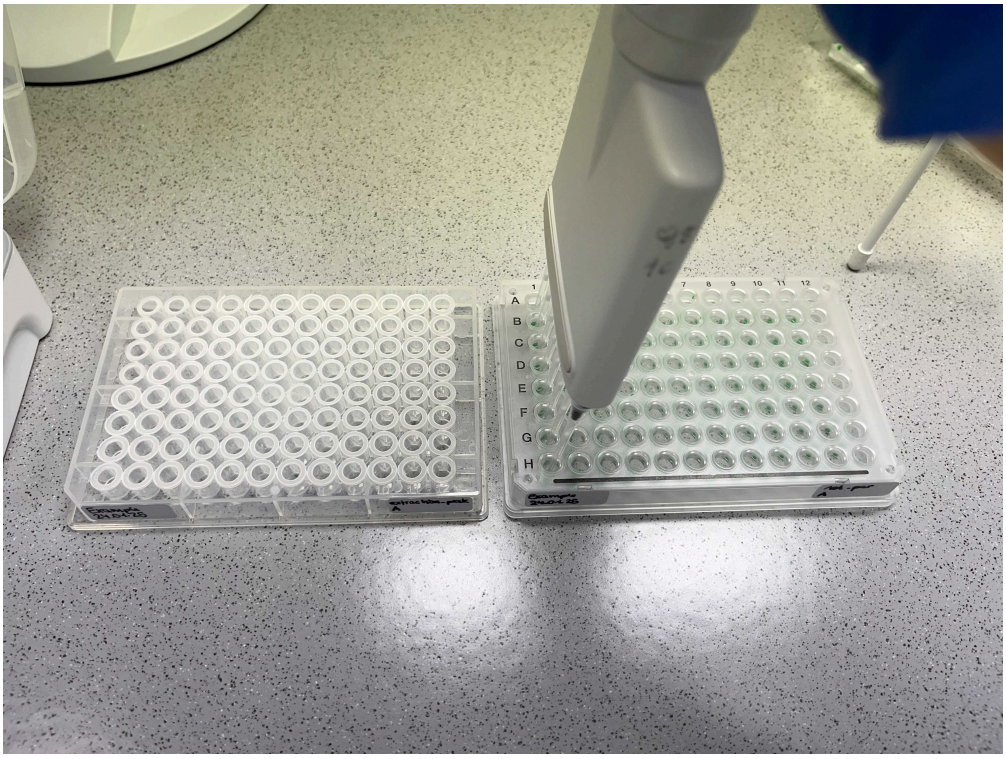
4m



Always maintain the order of samples (here top to bottom, left to right) for pipette tips, source plate and target plate (see below).



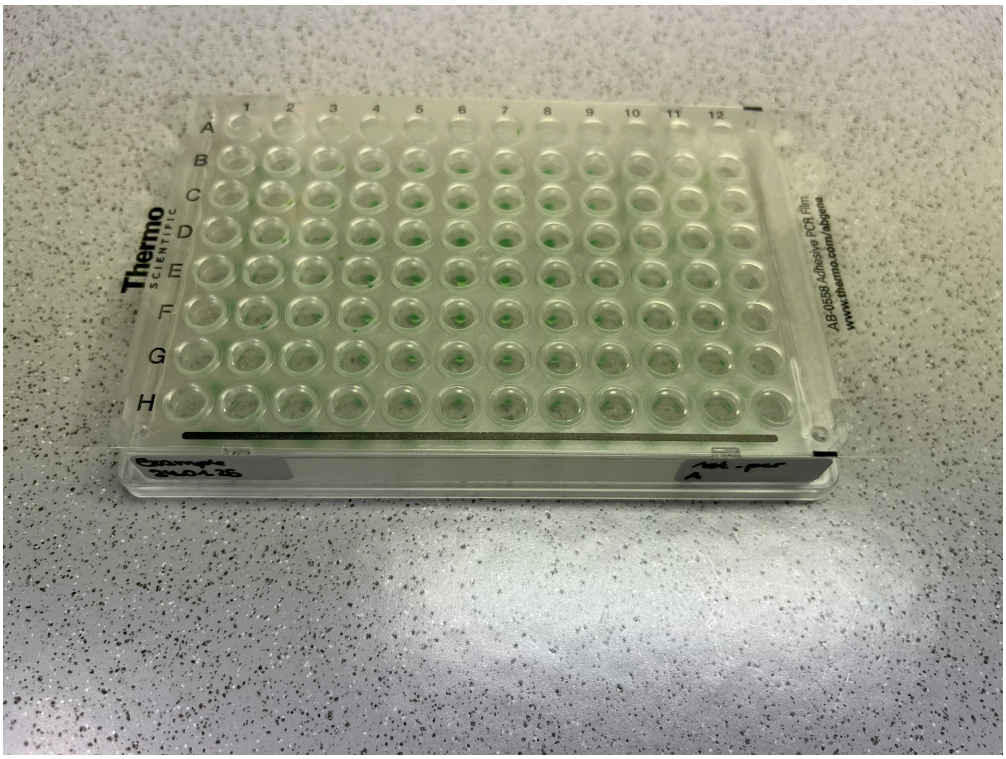
Use 1 µL of each sample (DNA extract).



Fill the DNA extracts in the respective wells (same order on each plate).

7 Close the Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601 with PCR foil, DNase-/RNase-free **Sarstedt Catalog #**95.1993. Make sure that each well is properly sealed.

1m



Sealed PCR plate.

7.1 Close the extraction plate with PCR foil. Make sure each well is properly sealed. 1m

8 Briefly vortex and shortly spin down the sealed Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601 . 1m

PCR 2h 2m

9 Place the sealed Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601 in a Thermocycler and close it properly. 1m

9.1 Depending on the Thermocycler, a lid or pad should be placed on the PCR plate before closing the Thermocycler. 1m

10 Enter/select the correct program (depending on used primers and chemicals) and start the polymerase chain reaction. 2h

Note

PCR program for fwhF2/fwhR2n:

Step 1: 95°C for 5 minutes (initial denaturation)
Step 2: 95°C for 30 seconds (denaturation)
Step 3: 58°C for 90 seconds (annealing)
Step 4: 72°C for 30 seconds (elongation)
--- repeat for 20 cycles ---
Step 5: 68°C for 10 minutes (final elongation)

Note

For samples with low template amounts (e.g. eDNA from water), also 30 cycles of 1st step PCR can be used.

Storage

1m

11 Vortex and spin down the Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #**HSP9601 to use it for further processing, e.g. gel electrophoresis, and/or store it at 4°C (short-term) or -20°C (long term).

1m