Article	Reactions
SL-9130	1 ml, 100 rxn x 20µl
SL-9130-5ML	5x 1 ml, 500 rxn x 20µl
SL-9130-10ML	10x 1 ml, 1000 rxn x 20µl
SL-9130-20ML	20x 1 ml, 2000 rxn x 20µl
SL-9130-50ML	50x 1 ml, 5000 rxn x 20µl

Storage Conditions
Long-Term Storage
Master Mix at -20 °C
Short-Term Storage
Master Mix at 4 °C

primaDIRECT PCR

Ultra-Fast Direct-PCR

DESCRIPTION

The **primaDIRECT** 2x PCR Master Mix enables direct PCR for fast and reliable genotyping and other standard PCR applications.

In combination with our primaDIRECT High Performance Lysis Buffer (SL-9102), **samples of various origin** (tissue, cells, mouse tails, plant pieces) are lysed within 15 minutes using our proprietary buffer **without the need of** column- or magnetic-based **extraction procedures**.

This 2x PCR Master Mix does not contain loading dye.

FURTHER INFORMATION

For more information, please visit our website

https://www.steinbrenner-laborsysteme.de

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SAMPLE LYSIS (NOT INLCUDED) & PCR WORKFLOW

BEFORE YOU START

- The High Performance Lysis Buffer is not included and can be obtained seperately (SL-9102-20ML).
- **If you use your own lysis buffer system, please continue with step 2.**

STEP 1: SAMPLE LYSIS

1. Fill 50 - 100 mg of your sample in to a 1.5 mL reaction tube
    1. If using cells, fill 10^3 - 10^6 cells in to a tube, spin it down at max speed and remove the supernatant mediuam
2. Add 50-200 µl RTL buffer
3. Incubate at 56°C for 15-30 minutes
4. Incubate at 95 °C for 3-20 minutes to stop the enzymatic reactions
5. ***Optional:*** Spin down your sample and transfer the supernatant to a fresh 1.5 mL tube - recommended for long-term storage
6. Place your sample on ice while preparing the PCR reaction
7. Add 2 µl of your lysed sample to the PCR reaction mix.

LYSIS OF SAMPLE MATERIAL

- **The lysis will not completely degrade the sample - which is absolutely fine and does not have any influence on the PCR.**
- You can lyse various types of sample material such as cells, animal tissue, plant leaves, mouse ears or mouse tails.
- In general, use as little sample material for lysis as possible to reduce the amount of PCR inhibitors present in the reaction mixture.

STEP 2: PIPETTE PCR REACTION MIXTURE

Components	20 µl Reaction	Final Concentration
2x primaDIRECT Master Mix	10 µl	1x
Forward Primer	variable (e.g. 2 µl)	200 - 400 nM
Reverse Primer	variable (e.g. 2 µl)	200 - 400 nM
Lysed Sample	**1-2 µl**	-
Sterile Water	**adjust to 20 µl**	

STEP 3: RUN PCR

Step	Time	Temperature
Initial Denaturation	2 minutes	95 °C
35 - 40 cycles		
Denaturation	5 seconds	95 °C
Annealing	10 seconds	55 °C - 68 °C
Extension	variable, depends on fragment length **5 - 20 seconds per 1 kb** fragment length	72 °C

Please see pages 5 & 6 for faster PCR protocols

STEP 4: VISUALIZE ON GEL

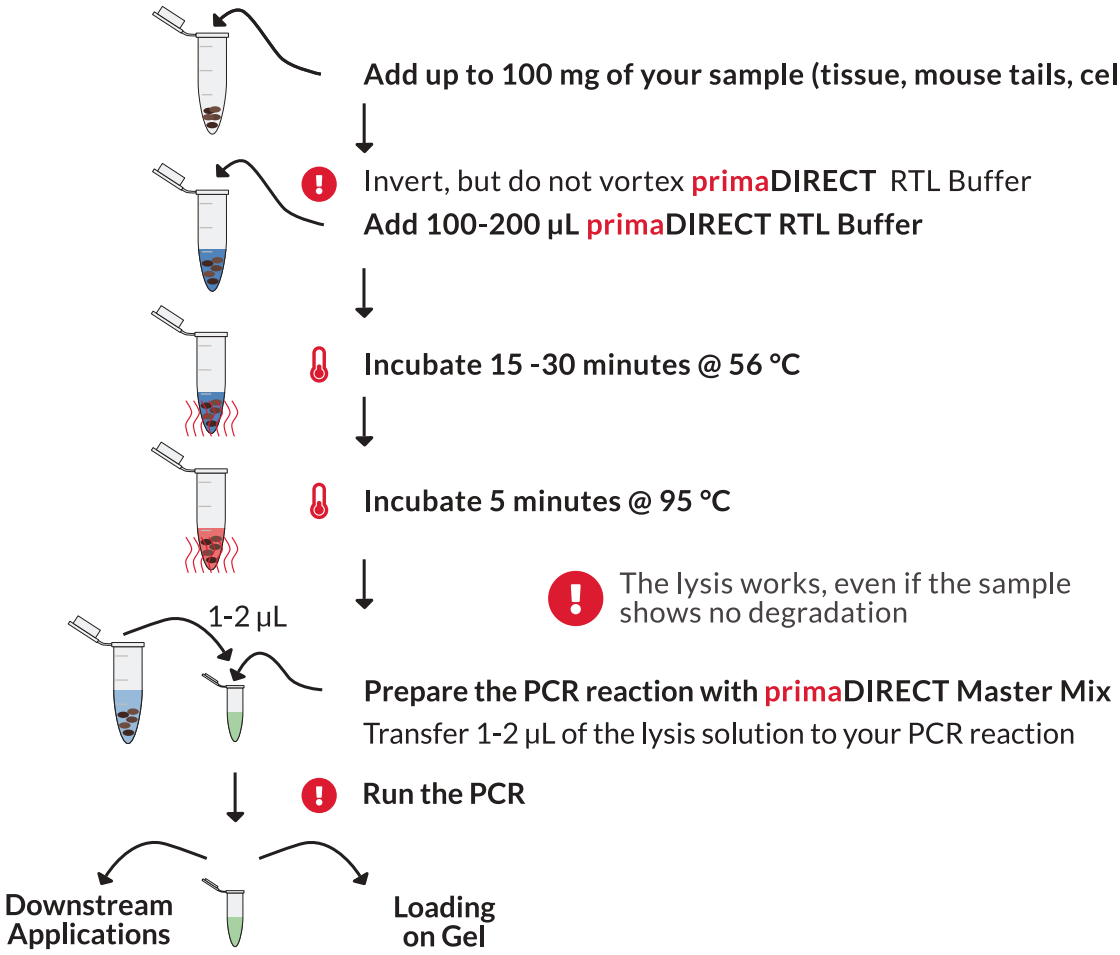
Transfer 20 µl to the agarose gel - **loading dye is required.**

NOTE

This **primaDIRECT** 2x Master Mix does not contain a loading dye. Additional loading dye is required for gel visualization.

GRAPHICAL WORKFLOW

LYSE + INACTIVATE PCR



STANDARD GENOTYPING PROTOCOL

BEFORE YOU START

- After thawing, please **invert the Master Mix tube 6-8 times.**
- **Do not vortex** the Master Mix to prevent damage of the enzyme.

NOTE

- Cycling conditions highly depend on the primer, probe, amplicon and input material and thus might need adjustments.
- However, standard cycling conditions can be applied for the majority of qPCR assays out-of-the box.

3-Step Protocol

Step	Time	Temperature
Initial Denaturation	1-3 minutes	92 °C - 95 °C
25- 40 cycles		
Denaturation	5 - 10 seconds	92 °C - 95 °C
Annealing	5 seconds	60 °C - **depending on primer**
Extension	**20 - 30 seconds**	72 °C

2-Step Protocol

Step	Time	Temperature
Initial Denaturation	1-3 minutes	92 °C - 95 °C
25- 40 cycles		
Denaturation	5 seconds	92 °C - 95 °C
Annealing/Extension Combined	**20 - 40 seconds**	60 °C - **depending on primer**

ULTRA-FAST GENOTYPING PROTOCOL

BEFORE YOU START

- After thawing, please **invert the Master Mix tube 6-8 times.**
- **Do not vortex** the Master Mix to prevent damage of the enzyme.

NOTE

- Ultra-fast Cycling conditions **highly depend on the ramping rate of your PCR cycler, primer, amplicon and input material** and thus might need adjustments.
- Ultra-fast cycling conditions can be applied for the majority of PCR assays out-of-the box, provided that your primer/probe sets do not show unspecific binding.

3-Step Protocol

Step	Time	Temperature
Initial Denaturation	60 seconds	92 °C - 95 °C
25- 40 cycles		
Denaturation	1-5 seconds	92 °C - 95 °C
Annealing	1-5 seconds	60 °C - **depending on primer**
Extension	**5 seconds**	72 °C

2-Step Protocol

Step	Time	Temperature
Initial Denaturation	60 seconds	92 °C - 95 °C
25- 40 cycles		
Denaturation	1 second	92 °C - 95 °C
Annealing/Extension Combined	**5-10 seconds**	60 °C - **depending on primer**