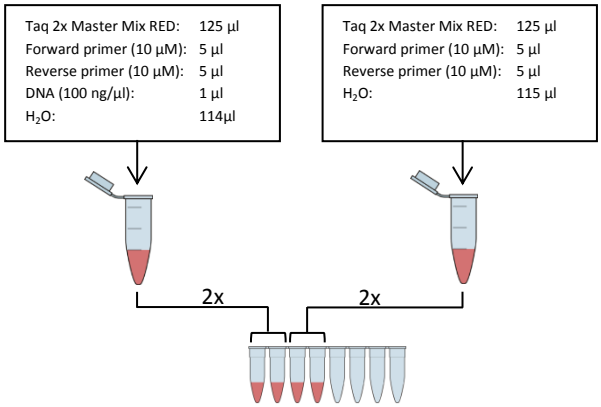
AMPLIQON
PCR ENZYMES & REAGENTS

Taq 2x Master Mix RED Evaluation guide

This guideline provides a protocol on how to set up and evaluate Taq 2x Mater Mix RED in a quick and simple way. It is very easy to use the Taq 2x Master Mix. Just add water, sample and primer, and PCR is ready to go. Even running the gel is easy since loading buffer is already included in the mix.

1) Prepare the PCR

Prepare a reaction mix that contains DNA and a reaction mix that does not contain DNA, as a negative control. Mix by flicking the tube without vortex. Pipet 50 µl of each reaction mixture into two separate PCR tubes.



2) Run the PCR

Use the PCR cycling protocol below when running the PCR tube strip in the PCR thermal cycler.

Phase	Time	Temperature	Cycles
Initial heating	5 min.	95 °C	1
Denaturation	30 sec.	95 °C	35
Annealing	30 sec.	60 °C*	
Elongation	30 sec.	72 °C	
Final elongation	4 min.	72 °C	1

* Apply the annealing temperature for the specific primer set.

3) Run the gel

Prepare a 1-2 % gel (dependent on target length). Distribute the PCR product directly onto the gel, without adding loading buffer. Add DNA ladder to the gel, to determine target length, and run the gel at e.g. 140 V.

4) Evaluate the results

The results should be within the specifications listed below in order to accept the mix. Use the table below to evaluate the mix.

Parameter	Specifications	Achieved?	
Band	Clear & distinct	Yes □	No □
Specificity	Correct length	Yes □	No □
Smear	No smear	Yes □	No □
NTC's	Negative	Yes □	No □
Accept mix?	"Yes" to all	Yes □	No □

Notes

Smear

If the gel has a smear of product throughout a lane, then either several unintended targets are being amplified or the DNA product is being degraded. Amplification of several unintended targets can often be overcome by optimizing the annealing temperature.

Influence of $MgCl_2$

$MgCl_2$ can promote a higher yield but too high concentrations can also promote unspecific amplification. Therefore, it is important to have the correct concentration of $MgCl_2$. When using Taq 2x Master Mix RED, the final $MgCl_2$ concentration is 1.5 mM, but more can be added if necessary.

Gradient PCR thermal cycler

Different primers have different optimal annealing temperatures. Therefore in order to gain the best results it can be required to optimize the annealing temperature. This can be done by using a PCR gradient thermal cycler

Avoid contamination

Three ways to avoid contaminations:

1) Work carefully and use filter tips
2) Employ PCR grade water
3) Have separate workspaces for PCR reaction setup, PCR thermal cycling and gel electrophoresis.

If these parameters are followed, most contamination issues can be avoided.

Sensitivity

If it is wanted to study the mix more deeply, then the sensitivity of the mix can be measured.

This can be done by preparing a dilution series of gDNA from 20 ng to 0.1 ng. If working with cDNA then the dilution series could be extended to even lower concentrations.

Remember that the more sensitive the mix is, the more risk of unspecific amplification. Therefore it is important that the primers are designed correctly and that contamination is avoided.

Sensitivity is also highly dependent on other parameters, such as the ones suggested in the table below.

Other Parameters

- Primer design
- Annealing temp
- Primer concentration
- Sample concentration
- Inhibitor contamination
- Choice of controls
- $MgCl_2$ concentration