LOEWE®

PCR – a simple experiment?

PCR experiments follow a simple work routine and thus it is tempting to believe that these types of experiments are easy to do. Especially workers who are dealing with large samples for years sometimes overlook the accompanying working protocols because they knew already everything. Thus it can happen that an experiment fails or does not provide clear results. This short introduction will draw your attention to some important facts and will assist you to solve potential problems.

Sampling

Correct sampling is in fact the key to successful PCR testing. Thus, by designing the experiment, please reconsider the following questions:

- Did you choose the right season and temperature for sampling?
- Did you choose the right parts of the plants (leaves, blossoms, roots, stems, seeds, tubers)
- What about the age of the plants?
- Did you take mixed samples or individual samples form different parts of the plant?
- Are enrichment steps necessary (culture of bacteria or fungi on selective or enrichment media)?
- Is transportation and storage of samples well organized (short time, cool environment, refrigerator, no storage or transport during weekend or holidays)

Sample preparation for PCR

DNA and RNA isolation protocols are used to extract the desired nucleic acid, which is then used as template for the PCR amplification. It is possible that co-enrichment of contaminants during the extraction causes inhibition of the PCR.
Several protocols are available for different plant types and plant tissues, which are especially adapted to avoid co-enrichment of potential contaminants during nucleic acid extraction. Commercial kits suggest adding additives such as polyvinylpyrrolidone or NaCl to the lysis solution if using lignified tissues. Please ensure that your nucleic acid isolation protocol is adapted for the tissue you intend to test. In some cases the DNA template solution can be diluted 1:10 to provide a sufficient inhibitor dilution. Tentative inhibition by the template could be tested by adding the template as an additive to any PCR reaction which is known give a positive result. Furthermore we recommend to measure the DNA concentration and purity using a spectrophotometer. An A260/A280 absorbance ratio of ~1.8 is accepted as pure DNA. Strong absorbents at 280 nm might result in a lower A260/A280 ratio, indicating co-isolation of contaminants during the DNA isolation protocol.

Dilution of Premix and Controls

Our premix consists of lyophilized pre-mixed dNTPs, as well as forward and reverse primers in optimized concentrations. The negative controls are made of lyophilized DNA isolated from a healthy plant, while the positive controls consist of DNA that produces an amplicon the same size as a ‘real’ positive sample would do. For all lyophilized reagents it is very important to:

1) spin down tubes for a few seconds at maximum speed
2) add water in an amount as described and pipette up and down, **do not vortex!**
3) leave on ice for 10-30 minutes
4) pipette again up and down to ensure complete solubilisation of the sample

Although the reagents are stable during delivery at room temperature, long term storage of the vials containing the premix and the controls **must be at -20°C**, even if water will be added at later.

Positive and Negative Controls

As with every experiment, for PCR it is also recommended to run a positive and a negative control next to the samples being analysed. Using our positive and negative control you can both ensure correct target amplification during the reaction cycles and rule out potential contamination of the PCR components. For instance, laboratories which run routinely diagnostic PCR on one specific target might be more prone to cross-contamination due to a higher probability of the DNA amplicon being 'around' in the laboratory. We recommend pipetting the PCR mastermix in a separate room/hood to the template and amplicon DNA preparation location and only then add the template to the mastermix. In general 'Good Laboratory Practices' and laboratory hygiene should be applied when conducting PCR analysis.

TROUBLESHOOTING

ERROR	CAUSE	SOLUTION
No PCR band	• Reaction component omitted	➢ Repeat reaction set up
	• Insufficient template	➢ Check DNA concentration, possibly repeat DNA extraction. Try using at least 10 ng DNA as template.
	• Unpure template	➢ Check DNA quality by measuring the ratio A260/A280, dilute template 1:10 or repeat DNA extraction
	• PCR thermocycler incorrectly programmed	➢ Check cycling program, in particular key parameters such as annealing temperature, elongation time and cycles. The suggested protocol might need to be adjusted to your cyler! **! The LOEWE® complete (RT-)PCR reaction kits contain a HOT Start DNA-Polymerase, which requires an initiation step at 95°C !**
	• Nuclease contamination on pipette tips or reaction tubes	➢ Use certified nuclease free working material
	• 5x PCR buffer or Premix not fully thawed when used	➢ Repeat reaction set up
Unspecific or multiple bands	Contamination with exogenous DNA	Use certified DNA free material, pipette mastermix and templates in separated dedicated areas