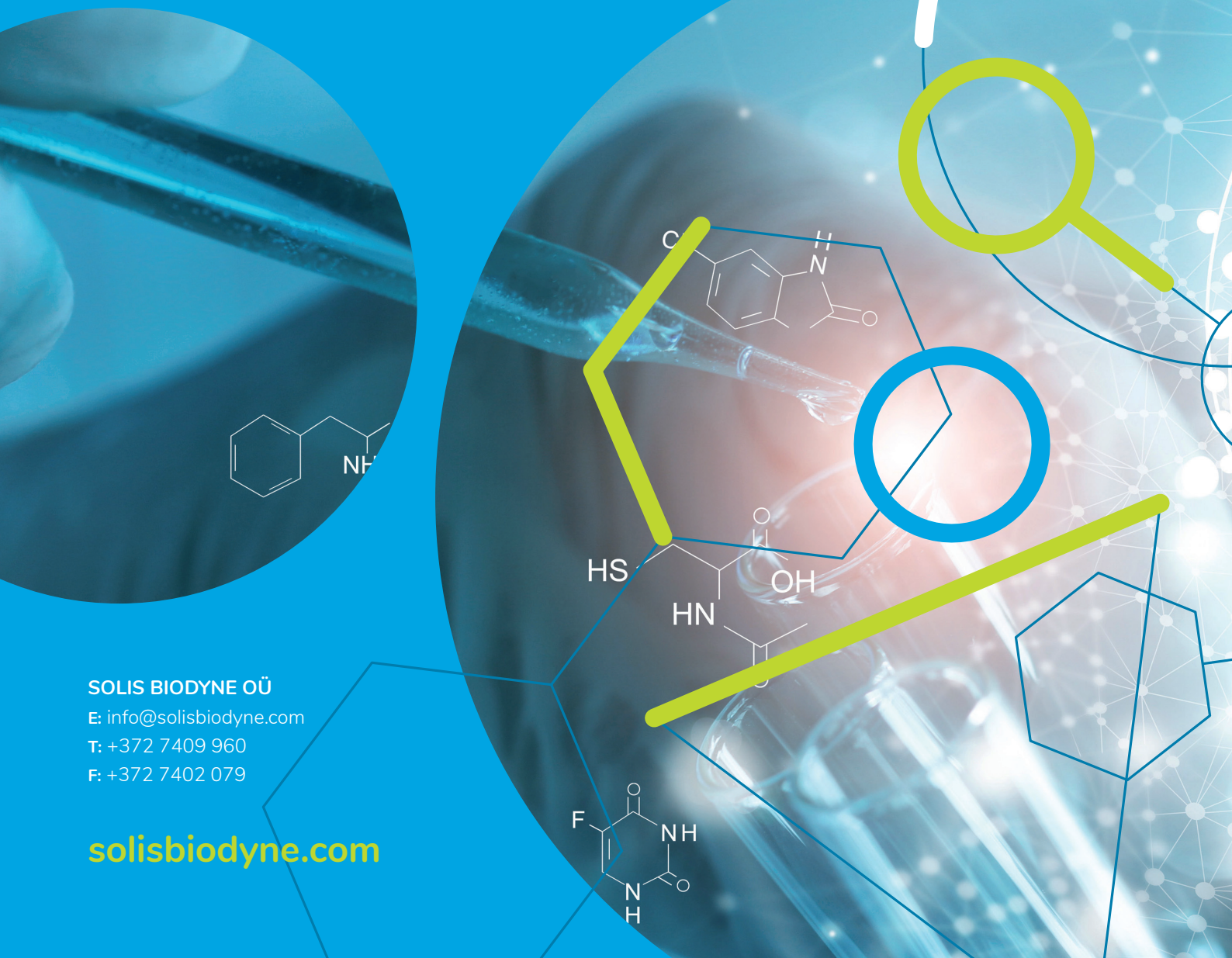
SOLIS BIODYNE

Multiplex PCR – maximize your throughput

Save time and money!



SOLIS BIODYNE OÜ
E: info@solisbiodyne.com
T: +372 7409 960
F: +372 7402 079

solisbiodyne.com

What is multiplex PCR?

First used as a method in detecting deletions in the dystrophin gene back in 1988[1], multiplex PCR has since made its way into many protocols across a variety of research areas. While still used in gene deletion analyses, multiplex PCR as well as qPCR is an incredibly useful tool in many applications, such as SNP genotyping, pathogen detection and food analysis to name just a few.

Multiplex allows for the researcher to detect multiple targets in a single reaction well. The prerequisite for this is using more than one primer pair for simultaneous target amplification and analysis.

Why to multiplex?

Save time and money

Multiplex PCR is a useful tool in any laboratory working with repeating samples and targets. Incorporating it into your workflow allows you to work through your samples faster while using less sample as well as consumables.

Multiplexing can be applied both in endpoint PCR as well as in real-time qPCR.
In endpoint PCR, gene targets are discriminated by amplicon size and detected typically via gel electrophoresis. The amplicon size may be limited by the properties of a DNA polymerase. Typical Taq DNA polymerases allow amplification of up to 5 kb fragments. With endpoint PCR generally more targets can be detected in one reaction compared to real-time qPCR.
In qPCR, the amplicon length is rather limited, typically between 100-200 bp, and the amplicons are detected in real-time using mostly target-specific hydrolysis probes (i.e TaqMan) or dsDNA intercalating dyes (i.e. SYBR® Green, EvaGreen®, or other similar dyes).

Multiplex qPCR with hydrolysis probes is highly adopted in the diagnostic sector, where it is used for detecting several pathogens as well as an internal control in the same sample in an extremely specific manner. Its time-saving, cost-efficient and increased reliability features make it highly beneficial for routine and high throughput experiments.

Each target gene must bind a differently labeled probe to set its amplification signal apart from the other targets in the same reaction, as each label emits fluorescent light at a different wavelength that is detected by specific channels in the qPCR cycler.

- Saves time
  - From pipetting: one 4-plex reaction versus four singleplex reactions
  - Frees up space on the plate and allows to get more data from one qPCR run
  - Reagents that enable fast cycling save even more time and money!
- Saves money
  - Less reagent used
  - Fit more samples on one plate
- Saves rare samples
  - No need to divide the sample between several reactions
- More reliable results
  - Targets of interest and positive control in the same reaction
  - UNG containing mixes reduce the risk of carry-over contamination

Considerations with multiplexing

Three key points to keep in mind

- Firstly, regarding primer design, researchers should make sure to validate new primers separately in a singleplex reaction to ensure they work efficiently. One should also keep in mind that primers should not have more than a 3°C difference in Tm. Primers should be experimentally checked for primer dimer formation.

- When it comes to probe design, researchers should make sure that each probe has a fluorescent label that is detectable in a different channel. One must also ensure probe label and quencher compatibility. With quenchers, it is important to note that some of them can change probe Tm significantly. Probe Tm should be 7-8°C higher than primer Tm.

- Finally, it is important to address qPCR cycler limitations. Namely, the number of targets per reaction is limited by the number of channels in the cycler. It must also be considered that in some systems one channel is reserved for signal normalization by using passive reference dyes such as ROX or Mustang Purple® (or their analogs), and the same channel cannot be used for normalization as well as target detection. Calibration requirements should be followed according to the manufacturer's guidelines.

Choosing the right tools

Using regular qPCR mixes in highly multiplexed assays carries a number of risks, such as late Ct values, low efficiency, low sensitivity and low fluoresence. As regular qPCR mixes do not have sufficient amount of components for amplifying more than 2 targets simultaneously, you are running the risk of getting false negative results. Using specifically designed multiplex mixes will greatly increase your success.

Solis BioDyne multiplex-ready probe-based qPCR Master Mixes:

- For **fast cycling** speed and up to 5-plex reactions:
  - SolisFAST® Probe qPCR Mix (no ROX)
  - SolisFAST® Probe qPCR Mix (ROX)
  - SolisFAST® Probe qPCR Mix (Purple)
  - SolisFAST® Probe qPCR Mix with UNG (no ROX)
  - SolisFAST® Probe qPCR Mix with UNG (ROX)
  - SolisFAST® Probe qPCR Mix with UNG (Purple)

- For **standard cycling** speed and up to 4-plex reactions:
  - HOT FIREPol® Multiplex qPCR Mix (no ROX)
  - HOT FIREPol® Multiplex qPCR Mix (ROX)
  - HOT FIREPol® Multiplex qPCR Mix (Purple)

No compromise on sensitivity

By using the right product designed for multiplexing, you can rest assured you will enjoy similar sensitivity to singleplexing with a greatly reduced workload.

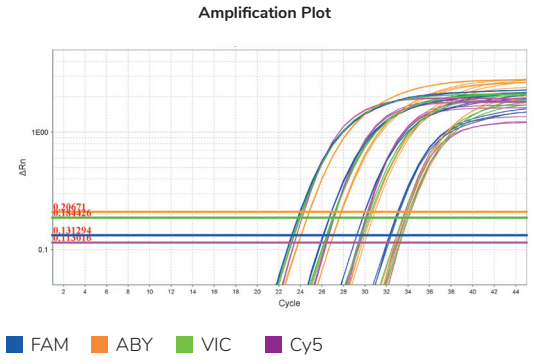


Figure 1. SolisFAST® Probe qPCR Mix (ROX) was used in a 4-plex probe-based qPCR with fourten fold serial dilutions of human gDNA (40 ng –40 pg per reaction, three replicates at each concentration). qPCR was performed on a QuantStudio™ 6 Flex qPCR cycler (Applied BioSystems™) using ROX dye for normalization.

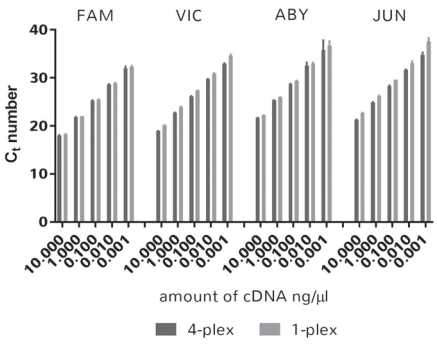


Figure 2. HOT FIREPol® Multiplex qPCR Mix (Purple) was used to perform 4-plex or single-plex probe-based qPCR with five tenfold serial dilutions of human cDNA (cDNA concentrationranges from 200 ng to 20 pg per reaction). Reactions were performed with Applied BioSystems™ QuantStudio™ 6 cycler using Purple dye for normalization.

Real-life applications

Making a difference every day

The scientific community is using multiplex on a daily basis to gain valuable insight into the world's most crucial issues.

In diagnostic tests requiring multiple different targets to be detected, multiplexing offers a way to increase efficiency. Often, quick and specific differential diagnosis is needed to confirm or rule out a number of potential pathogens responsible for a specific infection. Combined with reverse transcription, multiplex PCR and qPCR are also applicable in detecting several viral pathogens with RNA as a genetic material[2]. An example would be upper respiratory infections, where a number of viruses (eg. rhinovirus, influenza virus, human coronavirus) can cause similar and non-specific symptoms.

Malaria is a disease that is relevant in many parts of the world. Multiplex qPCR is a method that has shown its superior performance in achieving high-throughput screening of the disease[3]. Additionally, its reduced cost makes it a desirable choice for epidemiological and surveillance studies[4].

A fast differentiation between bacterial and viral pathogens is also needed for an accurate treatment plan and improved patient outcome for patients with pneumonia[5]. Smaller organ groups, such as eyes or joints, also suffer from infections of different origins and a correct diagnosis is crucial for applying the correct therapy. To illustrate, Chlamydia trachomatis, herpes simplex virus (HSV) and adenoviruses are the common causes of keratoconjunctivitis and a multiplex PCR reaction enables differentiation between them from just one conjunctival swab sample. The same principle applies to many other sample types, such as saliva, blood and urine samples and their most common pathogens.

Many veterinary laboratories also benefit from multiplex PCR tests, as different bacterial, viral, fungal and parasitic infections are no strangers to the zoological world. In the veterinary sector, diagnostic laboratories are often geographically far from the barns and stables, so samples often need to be shipped to testing facilities. Therefore, taking additional samples later may not be an option. Again, multiplex PCR enables the detection of various pathogens and markers from the same sample to get to the right diagnosis as fast as possible.

Multiplex PCR can be useful in many different fields. When working with plants, cell cultures, laboratory animals, microorganisms, diagnostics or forensics, consider your options to make your road to data faster and more effective.

Here at Solis BioDyne, we feel honored to be able to do our part and have our products as the tools of choice for key areas such as cancer research[6] among others.

About Solis BioDyne

Your experts in PCR

Solis BioDyne has been developing and producing life science reagents since 1995, having become one of the leading reagent suppliers in Europe today. High standards for production and service have made Solis BioDyne a trusted trademark worldwide. Our DNA polymerases, PCR Master Mixes, qPCR Mixes and reverse transcription reagents are used by a quickly growing number of customers across the globe, including top research institutes and biotech-companies. Solis BioDyne has partners in both private and state sectors, with cooperation projects ranging from OEM production to scientific research.

References:

1. Chamberlain, J. S., Gibbs, R. A., Ranier, J. E., Nguyen, P. N., & Caskey, C. T. (1988). Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic acids research, 16(23), 11141–11156. https://doi.org/10.1093/nar/16.23.11141
2. Kováčová, V., Boršová, K., D Paul, E. et al. (2021). Surveillance of SARS-CoV-2 lineage B.1.1.7 in Slovakia using a novel, multiplexed RT-qPCR assay. medRxiv 2021.02.09.21251168; doi: https://doi.org/10.1101/2021.02.09.21251168
3. Schindler, T., Deal, A.C., Fink, M. et al. (2019). A multiplex qPCR approach for detection of pfhrp2 and pfhrp3 gene deletions in multiple strain infections of Plasmodium falciparum. Sci Rep 9, 13107. https://doi.org/10.1038/s41598-019-49389-2
4. Kamau, E., Alemayehu, S., Feghali, K. C., Saunders, D., Ockenhouse C. F. (2013). Multiplex qPCR for Detection and Absolute Quantification of Malaria. PLOS ONE 8(8): e71539. https://doi.org/10.1371/journal.pone.0071539
5. Roisin, S., Huang, T. D., de Mendonça, R. et al. (2018). Prospective evaluation of a high multiplexing real-time polymerase chain reaction array for the rapid identification and characterization of bacteria causative of nosocomial pneumonia from clinical specimens: a proof-of-concept study. Eur J Clin Microbiol Infect Dis 37, 109–116. https://doi.org/10.1007/s10096-017-3108-3
6. Athina Markou, Marifili Lazaridou, Panagiotis Paraskevopoulos, Shukun Chen, Monika Świerczewska, Joanna Budna, Andra Kuske, Tobias M Gorges, Simon A Joosse, Thomas Kroneis, Maciej Zabel, Peter Sedlmayr, Catherine Alix-Panabières, Klaus Pantel, Evi S Lianidou, Multiplex Gene Expression Profiling of In Vivo Isolated Circulating Tumor Cells in High-Risk Prostate Cancer Patients, Clinical Chemistry, Volume 64, Issue 2, 1 February 2018, Pages 297–306, https://doi.org/10.1373/clinchem.2017.275503

SOLIS BIODYNE

For further details and ordering please contact
info@solisbiodyne.com or call **+372 740 9960**